

AMENDMENTS TO THE CLAIMS

1. (Presently Amended) A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising treating the separated single stranded sequences with

(a) first and second primers ~~each capable of hybridising, which hybridize~~ to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridized to a complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity,

D¹ (b) third and fourth primers each having a degree of sequence homology with the particularly digestible regions of the first and second primers respectively whereby the third and fourth primers ~~are capable of hybridising, hybridize~~ to the 3'-binding regions of the first and second strands respectively,

(c) an enzyme having strand displacing polymerase activity,

(d) an enzyme having 5' double stranded specific exonuclease activity, ~~said enzyme (d) possibly being provided by enzyme (c) in the case where the latter also has the required exonuclease activity, and~~

(e) nucleoside triphosphates,

under conditions permitting hybridisation, exonuclease digestion and strand displacement polymerisation thereby producing an amplified amount of the first and second strands.

D² 2. (Currently Amended) A method as claimed in claim 1 wherein the complementary first and second nucleic acid sequences are generated *in situ* from a single stranded nucleic acid molecule.

3. (Previously Amended) A method as claimed in claim 1 wherein the digestion resistant region is provided by modified nucleotides or ribonucleotides.

4. (Original) A method as claimed in claim 3 wherein the modified nucleotides provide phosphorothiate linkages which provide the resistance to digestion by the exonuclease.

5. (Previously Amended) A method as claimed in claim 1 wherein the first and second primers each comprise 30 to 60 bases.

6. (Original) A method as claimed in claim 5 wherein the digestion resistant region is provided 15 to 25 bases from the 5' end of the first and second primers.

7. (Previously Amended) A method as claimed in claim 1 wherein the third primer is of a sequence corresponding to the sequence in the first primer on the 5' side of the digestion resistant region of that primer.

8. (Previously Amended) A method as claimed in claim 1 wherein the fourth primer is of a sequence corresponding to the sequence in the second primer on the 5' side of the digestion resistant region of that primer.

9. (Previously Amended) A method as claimed in claim 1 wherein the third and fourth primers comprise 12 to 30 bases.

10. (Previously Amended) A method as claimed in claim 1 wherein the 5' double strand specific exonuclease is T7 Gene 6 exonuclease.

11. (Previously Amended) A method as claimed in claim 1 wherein the strand displacing DNA polymerase is at least one of, 9°N polymerase, Klenow (exo⁻) polymerase, *Bst* polymerase, Vent (exo⁻) polymerase, or Deep Vent (exo⁻) polymerase, *Pfu* (exo⁻) polymerase, *Tth* polymerase, *Tfl* polymerase, *Taq* polymerase or *Bca* (exo⁻) polymerase.

12. (Previously Amended) A method as claimed in claim 1 wherein the steps of exonuclease digestion and strand displacing polymerisation are effectively separated by performing the two reactions separately by removal of enzyme between steps, or, under conditions which favour the action of one or other enzyme.

13. (Previously Amended) A method as claimed in claim 1 effected isothermally.

14. (Previously Amended) A method as claimed in claim 1 wherein the digestible regions of the first and second primers are of identical sequence and the third and fourth primers are identical to these sequences.

15. (Previously Amended) A method as claimed in claim 1 wherein the 5'-ends of the first, second, third and fourth primers are resistant to digestion by an exonuclease functioning as a single strand active exonuclease.

16. (Previously Amended) A method as claimed in claim 1 wherein the amplification occurs in the presence of further primers specific to other target sequences (multiplex amplification) or to all or some of the same target sequence (nested amplification).

17. (Previously Amended) A method as claimed in claim 1 wherein at least one of the nucleoside triphosphates provided as (e) of claim 1 is modified such that when it is incorporated in a growing nucleic acid chain it is resistant to digestion by the exonuclease.

18. (Previously Amended) A method as claimed in claim 1 wherein the nucleic acid is DNA.

19. (Canceled) ~~A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising the steps of~~

~~(i) forming a reaction mixture comprised of the separated single strands together with~~

~~(a) first and second primers each capable of hybridising to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridised to a complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity;~~

~~(b) third and fourth primers each having a degree of sequence homology with the particularly digestible regions of the first and second primers respectively whereby the third and fourth primers are capable of hybridising to the 3'-binding regions of the first and second strands respectively;~~

~~(c) an enzyme having strand displacing polymerase activity,~~
~~(d) an enzyme having 5' double stranded specific exonuclease activity, said enzyme~~
~~(d) possibly being provided by enzyme (c) in the case where the latter also has the required~~
~~exonuclease activity, and~~
~~(e) nucleoside triphosphates~~
~~(ii) effecting a reaction under conditions permitting hybridisation, exonuclease~~
~~digestion and strand displacement polymerisation thereby producing an amplified amount of the~~
~~first and second strands.~~

20. (Currently Amended) A method of amplifying complementary first and second nucleic acid sequences each of which has a binding region at its 3' end, the method comprising treating the separated single stranded sequences with

D³ (a) first and second primers ~~each capable of hybridising~~, which hybridize to the 3'-binding regions of the first and second strands respectively and each including remote from its 5'-end a digestion resistant region which, with the primer hybridised to a complementary 3'-binding region, allows only partial digestion of the primer by the enzyme (d) having 5'-double strand specific exonuclease activity,

(b) third and fourth primers each having a degree of sequence homology with the particularly digestible regions of the first and second primers respectively whereby the third and fourth primers ~~are capable of hybridising~~ hybridize to the 3'-binding regions of the first and second strands respectively,

(c) an enzyme having strand displacing polymerase activity,

(d) an enzyme having 5' double stranded specific exonuclease activity, ~~said enzyme~~
~~(d) possibly being provided by enzyme (c) in the case where the latter also has the required~~
~~exonuclease activity, and~~

(e) nucleoside triphosphates which are modified such that when it is incorporated into a growing nucleic they are resistant to digestion by the exonuclease under conditions permitting hybridisation, exonuclease digestion and strand displacement polymerisation thereby producing an amplified amount of the first and second strands.

D4
21. (Previously Added, Currently Amended) The method of claim 1 wherein the third primer ~~is capable of hybridizing~~ hybridizes to the 3'-binding region for the first primer which is complementary to the 5'-side of the digestion resistant region of the first primer.

22. (Previously Added, Currently Amended) The method of claim 1 wherein the fourth primer ~~is capable of hybridizing~~ hybridizes to the 3'-binding region for the second primer which is complementary to the 5'-side of the digestion resistant region of the second primer.

23. (Previously Added) A method as claimed in claim 1 wherein the 5'-ends of the first, second, third and fourth nonhybridized primers are resistant to digestion by 5'-double strand specific exonuclease.

24. (Previously Added) A method as claimed in claim 1 wherein the 5'-ends of the first, second, third and fourth primers incorporate modified nucleotides which are resistant to digestion by 5'-double strand specific exonuclease.

D5
25. (New) The method of claim 1 wherein the enzyme having strand displacing polymerase activity is also the enzyme that provides the 5' double stranded specific exonuclease activity.

26. (New) The method of claim 20 wherein the enzyme having strand displacing polymerase activity is also the enzyme that provides the 5' double stranded specific exonuclease activity.